J. Physiol. (1969) **202**, 645–660
With 7 text-figures
Printed in Great Britain

STIMULATION OF ADRENAL GLUCOCORTICOID SECRETION IN MAN BY RAISING THE BODY TEMPERATURE

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(*Received* 30 *December* 1968)

SUMMARY

1. Plasma cortisol and corticosterone concentrations increased significantly in eleven resting, unacclimatized subjects after 2 hr exposure to an ambient temperature of 46° C dry bulb, 36° C wet bulb and in two subjects investigated by controlled elevation of body temperature in a hyperthermia test-bed.

2. In the same experiments the urinary excretion of 17-hydroxycorticosteroids (17-OHCS) estimated as the 11-oxy and 11-deoxy fractions did not differ significantly in hot and in control conditions.

3. Following an initial fall in plasma cortisol concentration during the first hour of heat exposure, cortisol levels increased in the second hour when body temperatures exceeded a 'critical' level of 38·3° C. Two acclimatized subjects did not attain this body temperature even after 2 hr heating and showed no increase in plasma glucocorticoid levels.

4. Sweat collected in arm bags, or by suction, in controlled hyperthermia experiments contained negligible amounts of cortisol (0·34–1·70 μg/100 ml. sweat).

5. Changes in plasma cortisol specific activity after intravenous injection of 1,2[^{3}H]cortisol indicated that the raised plasma concentration was brought about by increased adrenal secretion, though this was accompanied by more rapid removal of cortisol from the circulation in hot conditions. Excretion of tritium by the kidney was not significantly altered. It was not possible to determine whether changes in liver function contributed to the elevation of the plasma cortisol level but it was found that a larger proportion of cortisol was oxidized to cortisone, or to a metabolite closely resembling cortisone, in the heat.

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INTRODUCTION

Current evidence suggests that there is a causal relationship between the suppression of body heat production during prolonged exposure to high temperature conditions and reduction in the activity of the pituitary-thyroid-adrenal system (Macfarlane, 1963; Collins & Weiner, 1968). It is clear, however, that thermogenesis is influenced in quite the opposite way during acute elevation of body temperature which induces an increased oxygen usage and raises thyroid activity (Collins & Weiner, 1968). In the anaesthetized dog, hyperthermia is found to increase the level of 17-OHCS in adrenal venous and peripheral blood plasma (Richards & Egdahl, 1956). Small, but significant increases in plasma cortisol level have also been reported in the unanaesthetized dog with elevation of ambient temperature and with anterior hypothalamic pre-optic warming (Chowers, Hammel, Eisenman, Abrams & McCann, 1966). In similar experiments on the goat (Andersson, Gale, Hökfelt & Ohga, 1964) there were large fluctuations in blood cortisol levels which could not be related consistently to the effects of cooling or warming the heat-loss centre.

Many previous studies have been made of the changes in urinary excretion of adrenocorticosteroids during short-term heat exposure in man (e.g. Hellmann, Collins, Gray, Jones, Lunnon & Weiner, 1956; Robinson & MacFarlane, 1958; Streeten, Conn, Louis, Fajans, Seltzer, Johnson, Gittler & Dube, 1960), but these give uncertain indices of pituitary-adrenal activity. Human plasma 17-OHCS levels have been shown to increase during short periods of heating, though only in experiments which have also involved physical effort (Falbriard, Muller, Neher & Mach, 1955) or hypoxia (Hale, Sayers, Sydnor, Sweat & van Fossan, 1957). The investigations described in this paper, some of which have previously been reported (Collins, Few, Forward & Giec, 1968), were made in order to assess the effect on pituitary-adrenal activity of raising body temperature by acute exposure to severely hot conditions and by controlled hyperthermia (Fox, 1967) in resting subjects. A supplementary study was made of cortisol metabolism in high temperature conditions using tritium-labelled cortisol. A method of high specificity for estimating plasma cortisol and corticosterone (J. D. Few & T. J. Forward) is also described.

METHODS

Twelve male subjects, six members of the laboratory staff and six student volunteers, were studied for 24 hr periods on 2 separate days designated 'control' and 'heat' days respectively. The experimental procedures were fully explained to all the subjects beforehand and each willingly gave consent to his participation. Six subjects were studied in control conditions before the heat and six studied in the heat before the control day, usually with an

interval of 1 week between for each subject. The students were familiarized with the protocol before the experiments but had not previously undergone hot-room procedures. In all experiments, consecutive 3 hr urine collections were made at 09.30, 12.30, 15.30 and 18.30 hr and the 24 hr collection was completed with a 12 hr collection at 06.30 hr the following day. Urine samples were treated with chloroform and stored deep-frozen.

1st series (*eight subjects*). Peripheral venous blood samples (20 or 50 ml.) were taken at 09.30, 11.00, 12.30 and 15.30 hr on control and heat days, the plasma being immediately separated and frozen.

On the heat day, the subjects rested in a cool environment from 09.15 to 10.00 hr before entering the heat chamber. They were then exposed for 2 hr (10.00–12.00 hr) to a severely hot environment (46° C dry bulb, 36° C wet bulb, 150 ft. per min air movement) in a normal sitting position. No restriction was placed on the intake of fluid during heating. The second blood sample (11.00 hr) was taken after 1 hr heat exposure and the third (12.30 hr) within 30 min of leaving the chamber. The expected mean tolerance time for resting, unacclimatized subjects under the climatic conditions used in these experiments is 2 hr 10 min (Provins, Hellon, Bell & Hiorns, 1962). Only one subject had to be withdrawn from the heat after 1½ hr, and one subject collapsed on completion of the 2 hr exposure. Pulse rate, skin temperatures and oral and tympanic temperatures were continuously recorded and sweat losses calculated from body weight losses measured at 30 min intervals. Sweat samples were collected in an armbag every 30 min for the purpose of estimating adrenocorticosteroid losses in sweat.

2nd series (*six subjects*). The second series of experiments was made in order to study secretion rate and distribution of glucocorticoids during heat exposure. The same experimental procedure was followed as in the first series but with the following modifications: (i) 2–3 μc 1,2[^{3}H]cortisol was injected intravenously in 10 ml. NaCl (0·9 %, w/v) at 09.30 hr before entering the hot chamber. This amount of radioactivity was used after consultation with the M.R.C. Isotope Advisory Panel. Blood samples were then taken at 10.00, 10.30, 11.00, 11.30 and 12.00 hr during the period of heating and at 12.30, 14.00 and 15.30 hr after heating. Control day samples were taken at corresponding times. (ii) An additional urine collection was made at 24–48 hr.

Controlled hyperthermia (*two subjects*). In two subjects, the relationship between body temperature and the pituitary-adrenal response was studied in more detail using the controlled hyperthermia test-bed equipment (Fox, 1967). With this method the subject's body temperature could be elevated rapidly and maintained for a given period at any given body temperature level. After the subject had been first dressed in a polyvinyl chloride (P.V.C., I.C.I.) vapour-barrier suit he was wrapped in the hot-air distributing layer, covered with a further insulative layer and allowed to rest on the test bed for 30 min before the experiment began. Body temperature, measured by a thermistor in each auditory meatus (with the thermistor placed close to but not in contact with the tympanic membrane), was elevated quickly (usually within 15 min) to the selected target temperature and then maintained at that temperature for at least 30 min. Blood samples were taken at 15 min intervals throughout this procedure. Sweat was collected continuously by suction applied to the inner impermeable suit.

Chemical methods

Urinary 17-OHCS. These were measured by the method of Few (1968).

Plasma cortical and corticosterone. To 5–10 ml. plasma were added [^{14}C]cortisol and [^{3}H]-corticosterone (approximately 5000 c.p.m. each); the total volume was made up to 20 ml. with distilled water, 0·5 ml. NaOH was added and the mixture was extracted with 80 ml. methylene chloride by gentle mechanical mixing for 30 min. The methylene chloride was filtered through a silicone treated Whatman No. 1 filter paper and passed through a column of silica gel (1 cm diameter, 3 g silica gel). The column was washed with 10 ml. 2 % methanol in methylene chloride and the corticosteroids were eluted with 10 ml. 10 % methanol in methylene chloride; this eluate was evaporated to dryness in a 15 ml. conical tube in a water bath at 45° C.

The dried residue was spotted on to 2 cm wide strips of Whatman No. 2 chromatography paper. Eight such strips, joined at one end, were obtained from a 23 cm wide sheet of paper. The two outer strips were used for reference steroids (20 μg each of cortisone, cortisol and corticosterone) and the remaining six strips were used for plasma extracts. The papers were equilibrated overnight and developed for 3 hr using the Bush C system (Bush, 1952). The reference steroids were located under the ultra-violet lamp and the corresponding areas of the strips bearing the plasma extracts were cut out and eluted with methanol.

The methanol eluates were evaporated to dryness in the vacuum desiccator and further purified by thin layer chromatography (TLC) using the technique described previously (Few & Forward, 1968). Ethyl acetate was used as the solvent for the cortisol fractions and ethyl acetate–toluene-ethanol (35:60:5) for the corticosterone fractions. Aliquots of the TLC eluate were taken for fluorimetry, and for liquid scintillation counting in order to assess the losses incurred during the purification.

Fluorimetry was carried out using a Locarte LFM/5 fluorimeter, equipped with a zinc lamp, and sulphuric acid ethanol reagent. For cortisol, 75% sulphuric acid and a development time of 20–30 min was used, and for corticosterone, 65% sulphuric acid and 50–70 min. With each set of plasma extracts quadruplicate sets of standards were prepared; 0·2 and 1·0 μg for cortisol and 0·02 and 0·1 μg for corticosterone. A calibration factor was calculated for the standards included with each batch, and from this the quantity of steroid present in the TLC eluate was calculated. By correcting for the method losses, indicated by the recovery of the radioactive standards, the concentration of cortisol and of corticosterone in the plasma samples was obtained.

In the experiments in which [^{3}H]cortisol had been administered, the above procedure was modified by also adding to the plasma samples approximately 1000 c.p.m. of 4-[^{14}C]cortisone and 5 μg inactive cortisone before extraction with methylene chloride. The cortisone zones from the paper chromatograms of these samples were eluted and the tritium and ^{14}C separately measured using the screening method of Okita, Kabara, Richardson & Le Roy (1957). It was thus possible to calculate the amount of tritium present as cortisone ('cortisone-tritium'). Similarly tritium and ^{14}C were also measured in the cortisol eluates from the thin layer chromatograms so that it was possible to calculate the amount of tritium present as cortisol ('cortisol-tritium') and hence the specific activity of the plasma cortisol.

Conjugated metabolites of cortisol. After extraction of the free steroids with methylene chloride, the diluted plasma was added to 5 volumes of 1:1 ethanol–acetone mixture. After standing at $-15°$ C for 30 min the precipitated proteins were removed by centrifugation and the supernatant was evaporated to dryness using a rotary evaporator and a bath temperature of 40° C. The residue was partitioned between 10 ml. methylene chloride and 10 ml. pH 4·5 acetate buffer. The organic layer was discarded and to the aqueous layer was added 50 μg each of tetrahydrocortisone (THE) and of tetrahydrocortisol (THF) and 10,000 units of β-glucuronidase. After incubating for 48 hr this mixture was extracted with ethyl acetate and the extracted steroids were chromatographed on paper in the Bush C system. The zones containing the THE and THF were cut out, by reference to guide strips that had been stained with blue tetrazolium, and eluted with methanol. Aliquots of the eluates were taken for liquid scintillation counting and for estimation of the recovery of the inactive carrier by means of the Porter–Silber reaction. It was then possible to calculate the amount of tritium present as THE and THF.

Cortisol secretion rates. Cortisol secretion rates were determined from 24 hr urine collections; very little tritium was excreted in the second 24 hr period. After hydrolysis of the urinary steroid conjugates with β-glucuronidase the corticosteroids were extracted with ethyl acetate and separated by paper chromatography in the Bush B5 system for 18 hr. THE and THF were located by staining guide strips with blue tetrazolium. The appropriate areas of the chromatograms were cut out and eluted with methanol. The eluted steroids were treated with sodium borohydride and sodium metaperiodate (Few, 1961) and the

resulting 11β-hydroxyaetiocholanolone (11-HE) was further purified by paper chromatography in the system toluene–iso-octane–methanol–water (80:120:160:40). After elution of the 11-HE, aliquots were taken for liquid scintillation counting and for quantitation by the Zimmermann reaction so that the specific activity of the urinary THE and THF could be calculated. The mean of the specific activities of the two metabolites was used to calculate the cortisol secretion rate as follows

$$\text{Secretion rate (mg/24 hr)} = \frac{\text{radioactivity injected (d.p.m.)}}{\text{specific activity (d.p.m./mg)}}.$$

Urinary tritium. 0·5 ml. urine was mixed with 10 ml. toluene-PPO-dimethyl POPOP scintillator and 6 ml. ethanol and counted at least twice either to 10,000 counts or for 100 min. Counting efficiency, which was in the range 7·5–8·5%, was determined by 'spiking' with standard [^{3}H]toluene.

RESULTS

Urinary 17-OH corticosteroids

The mean urinary excretion of 17-OHCS of eleven subjects during a heat-exposure day and a control day in Series 1 and Series 2 experiments are given in Table 1. The total 24 hr output of 17-OHCS and of the 11-oxy and 11-deoxy fractions did not differ significantly on heat and control days. There was similarly no statistically significant difference in 17-OHCS excretion in 3 hr urine collections made during the heat period (09.30–12.30 hr) and during the comparable time on the control day, although, as expected, urinary flow was lower in the heat. Previous studies (Hellmann *et al.* 1956) suggest that changes in adrenocortical function as the result of heating might be reflected in 17-OHCS excretion in the period immediately following heat exposure. In nine out of the eleven subjects, total 17-OHCS excretion increased slightly in the post-heat period (12.30–15.30 hr) as compared with the pre-heat (06.30–09.30 hr), ($P < 0{\cdot}02$), but these values were not significantly different from those obtained at 12.30–15.30 hr in control experiments.

On both heat and control days there was a consistent diurnal decrease in 17-OHCS excretion in the second 12 hr collection period (18.30–06.30 hr) as compared with the first (06.30–18.30 hr) and a marked increase in the ratio 11-deoxy:11-oxy 17-OHCS (steroid 11-oxygenation index) as has been reported elsewhere (Few & Collins, 1969).

Plasma cortisol and corticosterone

Hot-room experiments. Figure 1 illustrates the changes in plasma cortisol and corticosterone concentration which occur when an unacclimatized subject is exposed at rest to a climate of 46° C dry bulb, 36° C wet bulb. The results of similar experiments on eleven unacclimatized subjects together with control experiments on the same subjects are summarized in Table 2. During the first hour of heating there was a fall in plasma cortisol level ($P < 0{\cdot}05$) and after the second hour a highly significant

increase ($P < 0{\cdot}001$). Three and a half hours after leaving the hot room, cortisol levels had returned to normal.

Only one of the eleven subjects showed an increase in cortisol concentration by the end of 1 hr heating. In the remaining ten subjects the mean decrease in plasma cortisol after 1 hr heating was $5{\cdot}4 \pm 1{\cdot}2$ μg/100 ml., which was a significantly greater fall ($P < 0{\cdot}02$) than during the same period in control cool conditions, $0{\cdot}5 \pm 0{\cdot}7$ μg/100 ml.

TABLE 1. Urinary excretion of 17-OHCS in eleven unacclimatized subjects exposed to heat (H) for 2 hr (10.00–12.00 hr) and in the same subjects in cool conditions. (Mean ± S.E. of mean)

	11-oxy 17-OHCS (mg)	11-deoxy 17-OHCS (mg)
Control		
06.30–09.30 hr	$1{\cdot}29 \pm 0{\cdot}10$	$0{\cdot}34 \pm 0{\cdot}13$
09.30–12.30	$1{\cdot}50 \pm 0{\cdot}10$	$0{\cdot}42 \pm 0{\cdot}06$
12.30–15.30	$1{\cdot}45 \pm 0{\cdot}09$	$0{\cdot}46 \pm 0{\cdot}06$
15.30–18.30	$1{\cdot}59 \pm 0{\cdot}16$	$0{\cdot}46 \pm 0{\cdot}12$
18.30–06.30	$2{\cdot}76 \pm 0{\cdot}31$	$1{\cdot}02 \pm 0{\cdot}15$
24 hr 17-OHCS	$11{\cdot}29 \pm 0{\cdot}90$	
Heat		
06.30–09.30	$1{\cdot}31 \pm 0{\cdot}15$	$0{\cdot}30 \pm 0{\cdot}03$
09.30–12.30	(H) $1{\cdot}51 \pm 0{\cdot}12$	(H) $0{\cdot}37 \pm 0{\cdot}05$
12.30–15.30	$1{\cdot}84 \pm 0{\cdot}21$	$0{\cdot}41 \pm 0{\cdot}06$
15.30–18.30	$1{\cdot}56 \pm 0{\cdot}24$	$0{\cdot}39 \pm 0{\cdot}06$
18.30–06.30	$2{\cdot}99 \pm 0{\cdot}44$	$1{\cdot}10 \pm 0{\cdot}16$
24 hr 17-OHCS	$11{\cdot}78 \pm 1{\cdot}24$	

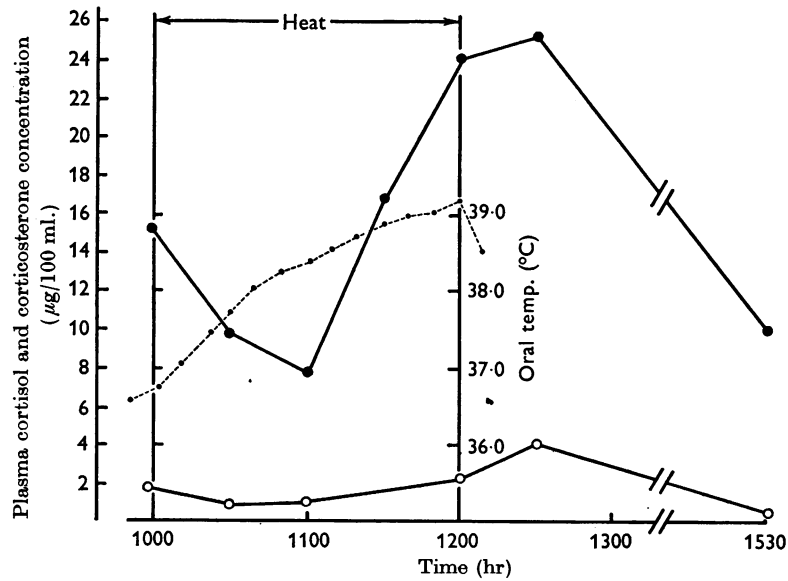

Fig. 1. Effect of 2 hr heat exposure (46° C dry bulb, 36° C wet bulb) on plasma cortisol concentration (●), plasma corticosterone concentration (○) and body (oral) temperature in a resting subject.

Plasma corticosterone concentrations were less than one tenth of the cortisol concentration, but in general the levels changed in parallel (Table 2).

Oral temperature increased progressively after 2 hr in the heat and in unacclimatized subjects reached 39·0–39·3° C (Fig. 1, Table 2). There was an initial marked increase in heart-rate in all subjects during the first 30 min followed by smaller increments in the second 30 min. At different stages in the second hour of exposure there were again periods of rapid increase, sustained in some subjects to the end of the experiment.

TABLE 2. The mean plasma cortisol and corticosterone concentration (± S.E. of mean) in unacclimatized and acclimatized (A) subjects during 2 hr exposure to heat (46°–36° C) and in control (cool) conditions

	No. of subjects	09.30 hr (pre-heat)	11.00 hr (after 1 hr heat)	12.00 hr (after 2 hr heat)	15.30 hr (3½ hr after heat)
Heat					
Cortisol	11	12·0 ± 1·4	7·9 ± 1·1	21·3 ± 3·0	11·8 ± 1·9
(μg/100 ml.)	2 (A)	9·4	4·6	3·6	5·6
Corticosterone	10	0·6 ± 0·1	0·7 ± 0·1	2·1 ± 0·4	0·5 ± 0·1
(μg/100 ml.)	2 (A)	0·5	0·3	0·3	0·2
Oral temperature (° C)	11	36·87 ± 0·04	38·30 ± 0·04	39·09 ± 0·5	—
	2 (A)	36·87	37·76	38·25	
Pulse rate	11	75 ± 4	114 ± 4	135 ± 5	—
(beats/min)	2 (A)	72	99	108	
Sweat rate	11	—	243 ± 22	326 ± 37	—
(g/m²/hr)	2 (A)		299	452	
Control					
Cortisol	11	9·5 ± 0·6	8·8 ± 0·8	7·9 ± 1·0	8·3 ± 1·6
(μg/100 ml.)	2 (A)	8·8	7·2	6·4	8·4
Corticosterone	10	0·6 ± 0·1	0·6 ± 0·1	0·8 ± 0·2	0·9 ± 0·1
(μg/100 ml.)	2 (A)	0·8	0·9	1·0	0·8

Two subjects, who were not deliberately acclimatized to heat, but who were at least partially acclimatized judging from their physiological performance in hot conditions (Table 2), reached a body temperature of only 38·25° C, and pulse rate of 120 beats/min at the end of the 2 hr. These were the only two subjects who did not show elevated plasma cortisol or corticosterone levels after 2 hr heating. Body temperatures and plasma steroid levels were similar in both of these subjects and a mean of the two results is given in Table 2.

Controlled hyperthermia. In the hot-room experiments described above (Table 2) it was apparent that in eleven unacclimatized subjects plasma glucocorticoid levels did not begin to rise until mean body temperature had reached 38·30 ± 0·04° C after 1 hr exposure, and that there was no elevation in the two acclimatized subjects in whom body temperature did not reach 38·3° C even after 2 hr. Experiments were therefore made on two subjects using the hyperthermia test-bed to investigate the effect of raising body temperature rapidly to predetermined levels in the critical zone. In

experiments on subject C, illustrated in Fig. 2, body temperature was raised quickly by 1·5 to 38·0° C on one occasion and by 2·1 to 38·6° C on another, the target being then maintained for 30 min. There was little increase in the plasma cortisol concentration at 38° C but a significant elevation at 38·6° C. A similar result was obtained on the second subject (F, Fig. 2) where the two target temperatures were attained in sequence in the same experiment.

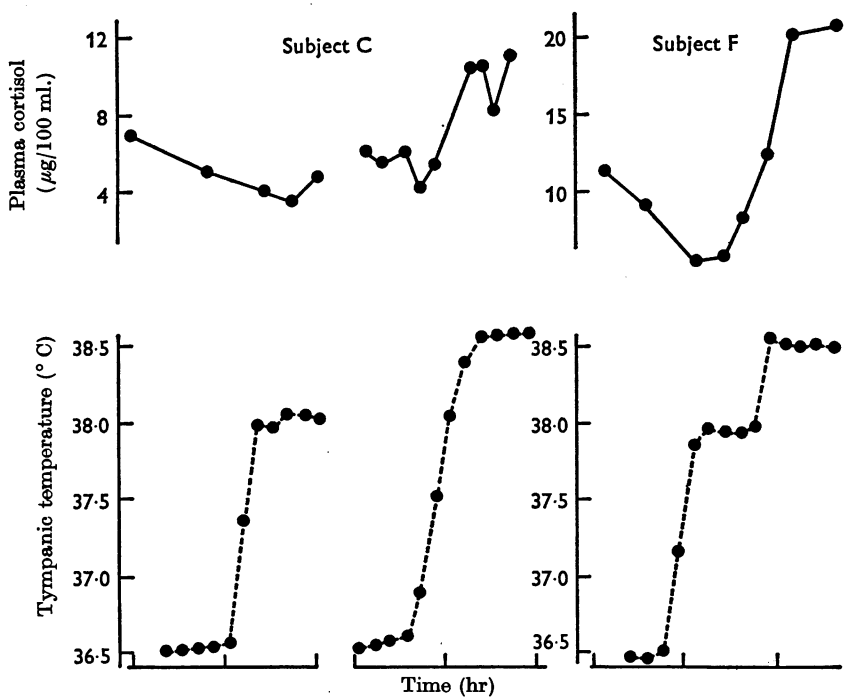

Fig. 2. Changes in plasma cortisol concentration (●—●) with elevation of body (tympanic) temperature (●----●) during controlled hyperthermia in two subjects.

Plasma cortisol concentrations plotted against aural (tympanic) temperature in controlled hyperthermia (Fig. 3) suggest that a critical temperature is reached at about 38·0° C in subject F and 38·3° C in subject C. Each point represents a body temperature maintained for at least 20 min. The experiments were conducted at approximately the same time of the day so that diurnal variations in resting plasma cortisol levels can be discounted.

Cortisol in sweat

During the first series of experiments, sufficient armbag sweat was collected (> 20 ml.) for the measurement of cortisol concentration to be attempted. In all cases the concentration of cortisol (range 0·7–1·7 μg/

100 ml.) was very much lower than that in plasma but as the plasma cortisol concentration was changing rapidly it was not possible to correlate the two values.

In the second series, less sweat was generally collected in the arm bags and in all except one experiment it was only sufficient for the measurement of tritium concentration. In all cases this was low (10–30 d.p.m./ml.). Assuming that the tritium content of arm-bag sweat is proportional to the

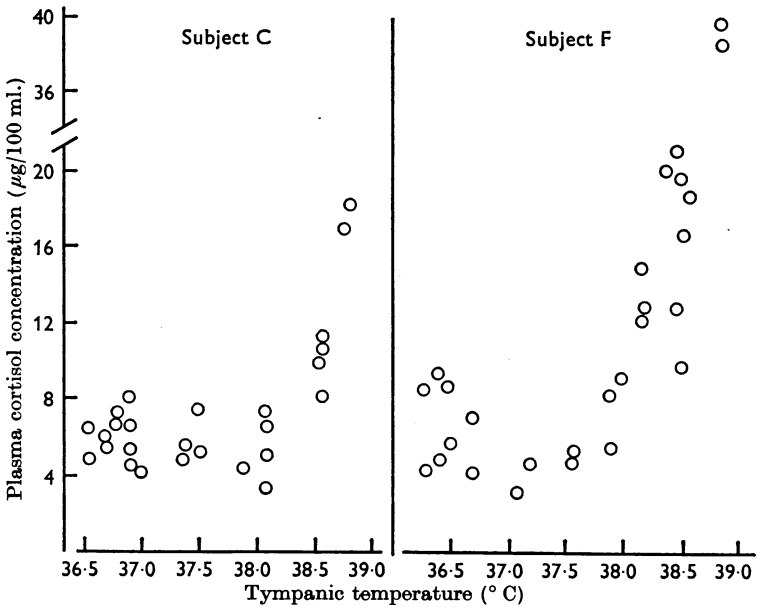

Fig. 3. Relation between plasma cortisol concentration and tympanic temperature in two subjects. Body temperature was kept constant for 20 min before a blood sample was taken.

total sweat loss of tritium no more than 2 % of the injected radioactivity is accounted for in sweat. From one subject, sufficient sweat was collected in the period 10.30–11.00 hr to permit the determination of cortisol concentration and cortisol-tritium. The cortisol concentration and specific activity were 1·3 μg/100 ml. and 2220 d.p.m./μg respectively; the corresponding values for plasma at 10.30 and 11.00 hr were, cortisol concentration 5·1 and 5·0 μg/100 ml. and specific activity 4450 and 2800 d.p.m./μg respectively. Total body sweat collected from two subjects in the hyperthermia test bed contained 0·48 and 0·34 μg cortisol/100 ml. The target body temperature in these experiments was 38° C and plasma cortisol levels did not exceed 12 μg/100 ml. Though this data is incomplete it suggests that loss of cortisol or of its metabolites in the sweat is quantitatively negligible.

Cortisol secretion and metabolism

Specific activity of plasma cortisol. All subjects in the second series of hot-room experiments received a tracer dose of tritium-labelled cortisol 30 min before entering the hot room. Plasma cortisol concentration and specific activity in heat and control experiments on one subject is shown in Fig. 4. During the period in the hot room when the plasma cortisol concentration was rising sharply there was a corresponding rapid fall in

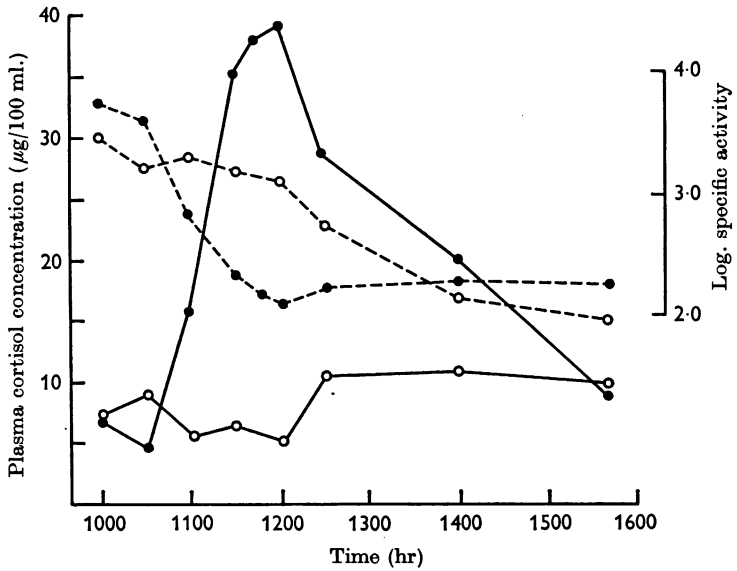

Fig. 4. Plasma cortisol specific activity following an intravenous injection (09.30 hr) of 2–3 μc of 1,2[^{3}H]cortisol in one subject exposed to heat (10.00–12.00 hr) and in the same subject in cool conditions. Specific activity in control (○----○) and heat (●----●); plasma cortisol concentration in control (○—○) and heat (●—●).

specific activity, and this was followed during the 4 hr after heating by a steady fall in plasma cortisol concentration while specific activity remained constant. In the control experiment with a fairly constant plasma cortisol level, specific activity fell gradually in a linear fashion over the 6 hr sampling period. It is suggested that these changes in plasma specific activity indicate (*a*) a specific increase in adrenal secretion of cortisol during heat exposure, and (*b*) suppression of adrenal secretion after heating, presumably as the result of the inhibitory action of raised plasma cortisol on the hypothalamic corticotrophin-controlling centre.

Rate of removal of cortisol from plasma. In Fig. 5 the rate of removal of tritium-labelled cortisol from plasma is compared in four subjects in the

heat and four in control. The mean plasma [^{3}H]cortisol concentration decreased more rapidly in the heat, to 37 % (range 29–45 %) of the initial level after 1 hr and to 20 % (range 12–32 %) after 2 hr in the heat, compared with 56 % (range 51–70 %) after 1 hr and 38 % (range 30–45 %) after 2 hr in cool conditions.

Cumulative urinary excretion of tritium. The increased rate of removal of cortisol from plasma in the heat cannot be attributed to changes in the urinary excretion of cortisol and its degradation products (Table 1). The

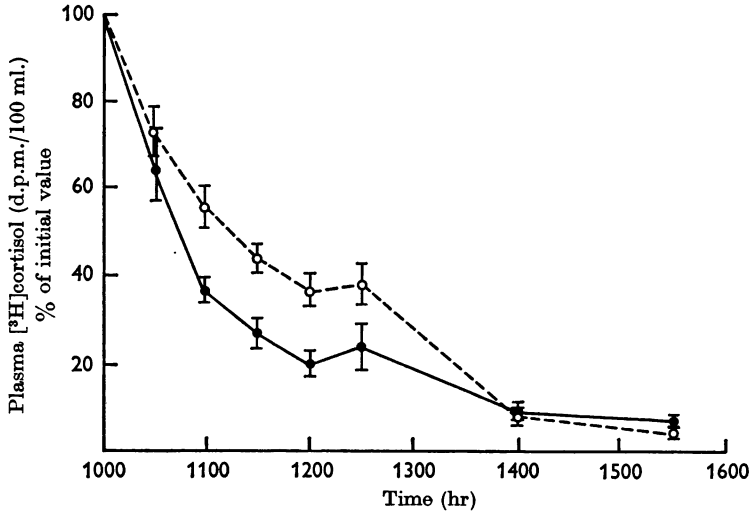

Fig. 5. Rate of removal of [^{3}H]cortisol from plasma expressed as a percentage (± S.E. of mean) of the initial plasma value at 10.00 hr in four control (○----○) and four heated (10.00–12.00 hr) (●—●) subjects. 2–3 μc 1,2[^{3}H]cortisol injected at 09.30 hr.

cumulative urinary loss of tritium after injection of [^{3}H]cortisol is in fact slightly less in heat-exposed than in control subjects (Fig. 6). In the first 6 hr from the beginning of heating, approximately 4 % less of the total tritium is excreted in heated subjects; the cumulative excretion then continues in parallel with the control for 48 hr.

Cortisol metabolism. Cortisol secretion rate was determined in six heat-exposed and five control subjects. The control values are in agreement with those found by Flood, Layne, Ramcharan, Rossipal, Tait & Tait (1961) for normal subjects. The small rise in the cortisol secretion rate shown by the heat-exposed subjects is significant at the 2 % level but neither the 11-oxy 17-OHCS excretion nor the ratio 11-oxy 17-OHCS : cortisol secretion rate, are significantly different from the control group (Table 3).

Estimation of cortisone-tritium in three heated and three control subjects showed that the ratio, cortisol tritium : cortisone tritium in plasma decreased

during the second hour of heating to below that in the control subjects (Fig. 7). THE tritium and THF tritium were measured in the plasma of two heated and two control subjects. One subject showed significantly higher levels during the second hour of heating, the other showed a similar distribution in time.

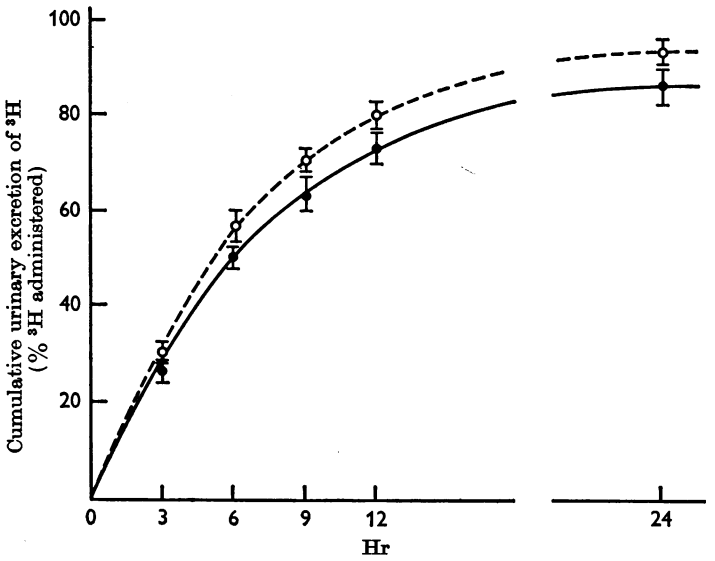

Fig. 6. Twenty-four hr urinary excretion of tritium after intravenous injection of tritium-labelled cortisol (at time 0). Mean values (±S.E. of mean) for five control subjects (○), and four subjects exposed to heat (●) between 0·5 and 2·5 hr.

TABLE 3. 24 hr urinary 17-OHCS excretion and cortisol secretion rate in six heat-exposed and five control subjects

	17-OHCS excretion		Cortisol secretion rate (mg/day)	11-oxy 17-OHCS × 100 cortisol secretion
	11-deoxy (mg/day)	11-oxy (mg/day)		
Heat	1·2	10·0	31·5	32·0
	2·4	9·5	22·4	42·5
	2·0	9·3	19·5	48·0
	2·4	12·0	33·4	36·0
	2·1	9·0	27·7	32·5
	3·4	15·2	28·8	53·0
Mean	2·2	10·8	27·2	40·7
Control	2·7	11·9	21·0	56·5
	2·2	7·4	19·5	37·7
	1·8	10·7	24·0	44·5
	2·3	9·0	17·5	47·0
	1·0	8·1	18·5	44·0
Mean	2·0	9·4	20·1	46·0

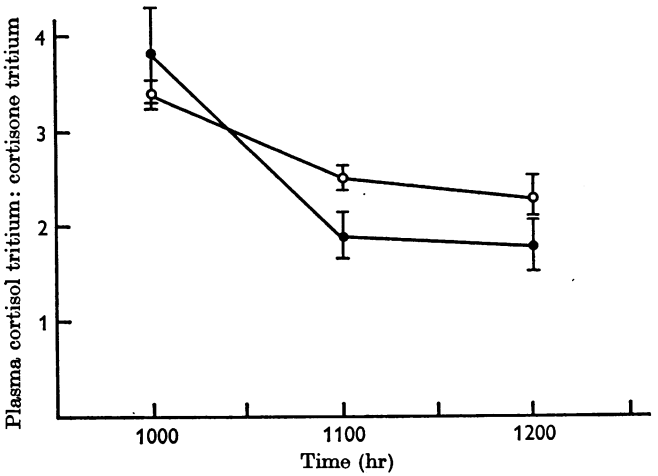

Fig. 7. Plasma cortisol tritium : cortisone tritium ratio in three heat-exposed (10.00–12.00 hr) (●) and three control subjects (○). Tritium-labelled cortisol injected at 09.30 hr.

DISCUSSION

A rise in body temperature, as induced by our methods, is an effective stimulus to the pituitary-adrenal system in man and plasma glucocorticoid concentration increases significantly when body temperature is raised by more than 1·5° C. Transient increases of two or three times the resting plasma cortisol level are not, however, reflected in corresponding changes in urinary 17-OHCS excretion. Seemingly, urinary 17-OHCS provide an unsuitable index of adrenal function in acute stress situations, a conclusion which confirms the findings of other authors (Thorn, Jenkins & Laidlaw, 1953). Urinary 17-OHCS output has, however, been reported to be raised in a subject brought to a state of collapse after a longer (4 hr) period of heat exposure (Robinson & MacFarlane, 1958) and in subjects submitted to 24 hr of hyperthermia (Beisel, Goldman & Joy, 1968).

The irreversible removal of cortisol from the blood is performed almost entirely by the liver which converts cortisol to a number of metabolites which are rapidly excreted by the kidney. Very little unchanged cortisol is excreted by the kidney in man. In the heat, cardiovascular, renal and body fluid adjustments are added factors which make more difficult the interpretation of blood levels and turnover of adrenocorticosteroids. A holistic view is therefore not possible without further detailed investigation of the fate and distribution of cortisol. In the steady state, liver function provides a self-regulation of adrenocorticosteroids with increased rate of removal as the plasma hormone level rises. Barlow, Agersborg & Keys (1956) have remarked on the importance of hepatic blood flow in con-

tributing to elevated plasma 17-OHCS with extreme hyperthermia in dogs. They found that the marked peripheral vasodilatation was accompanied by a rise in peripheral resistance probably as the result of intense vasoconstriction in the visceral region. Though little can be deduced about possible changes in hepatic removal of cortisol in the present experiments, of considerable interest is the apparent conversion of cortisol to cortisone in both the heated and the control subjects. While the reduction of cortisone to cortisol is well established (Bush, 1962, p. 365; Jenkins & Sampson, 1967), only Ichikawa (1966) has previously reported the oxidation of cortisol to cortisone. This latter author found a similar distribution of radioactivity between cortisol and cortisone in his normal subjects as we did in our control subjects. It is noteworthy that there is a marked difference in [^{3}H]cortisol:[^{3}H]cortisone between the control subjects and those exposed to the heat. It would, however, be necessary to demonstrate that such a difference was not solely due to the increased cortisol secretion observed in the heat-exposed subjects, before one could attribute this effect to the heat exposure. Further caution is necessary in the interpretation of these observations, as neither our own work nor that of Ichikawa (1966) provides a rigorous proof that this material is cortisone. Of course the difference between the heated and the control subjects would remain even if the material under consideration proved to be a hitherto unknown metabolite of cortisol.

Excretion of tritium by the kidney after administration of [^{3}H]cortisol is not increased by heat exposure and negligible amounts of radioactivity were measured in sweat. Neither of these routes of elimination, therefore, significantly affects the levels of plasma cortisol during acute heating for 2 hr. The sharp fall in plasma specific activity which accompanied the rising phase of plasma cortisol concentration in the heat clearly indicated an increased adrenal secretion rate. This was further confirmed by the daily secretion rate of cortisol measured by tritiated THF and THE in urine.

During the 2 hr exposure to a constant hot environment there were characteristically two phases in the plasma cortisol response, a decrease in concentration during the first hour followed by rising levels in the second hour. That a diurnal variation was mainly responsible for the first phase seems unlikely in view of the small diurnal change which would normally occur over the 2 hr period (Brown, Englert, Wallach & Simons, 1957) and the lack of a similar pronounced fall in the control experiments. Haemodilution is well known to be an early response to acute heating which occurs before there is appreciable loss of extracellular fluid by sweating (Bass & Henschel, 1956). The extent of the haemodilution is of the order of 5% or less and is not sufficient to account for the 30% reduction in plasma cortisol

level observed in the first hour of heating. The most reasonable explanation appears to be that the pre-heat cortisol levels were slightly raised due to the subjects' apprehension before going into the hot-room. No initial decrease in plasma cortisol level was reported in experiments on dogs when the pre-optic hypothalamic region was heated (Chowers *et al.* 1966), though this was accompanied by marked cutaneous vasodilatation.

The second phase in the response appears to be initiated when body temperatures become elevated above 38·3° C. It is worth noting that, in spite of the very high ambient temperature, none of the subjects reported any marked sensation of distress or panic, and most would have been willing to remain in the hot room for slightly longer than 2 hr. The two acclimatized subjects did not show raised plasma cortisol levels and their body temperature did not exceed 38·3° C even after 2 hr heat exposure. Furthermore, rapidly raising the body temperature from 36·5–38·0° C was ineffective and only at body temperatures above 38·0° C did increased stimulation of adrenal glucocorticoid secretion occur. In controlled hyperthermia studies it was also found that small increments in cortisol level could be obtained if the body temperature was held at 38° C for longer than 30 min but not at lower body temperatures even after 1 hr. Bligh (1966) has postulated that homeotherms may possess a secondary level of thermoregulation which operates at a definite upper and lower critical level and depends on the direct effects of temperature on the C.N.S. In the ruminant, abrupt changes in respiratory pattern (Findlay, 1957) and rise in moisture loss (Ingram, McLean & Whittow, 1961) occur at a critical deep body temperature of 40–41° C. It is suggested that the increased pituitary-adrenal response in man at body temperatures of 38·0° C and above depends on the stimulation of the hypothalamic centre regulating corticotrophin release when thermal equilibrium can no longer be maintained. Another expression of hypothalamic stimulation may be the sudden increase in heart rate observed when body temperature was elevated above this critical body temperature.

REFERENCES

ANDERSSON, B., GALE, C. C., HÖKFELT, B. & OHGA, A. (1964). Relation of pre-optic temperature to the function of the sympathico-adrenomedullary system and the adrenal cortex. *Acta physiol. scand.* **61**, 182–191.

BARLOW, G., AGERSBORG, H. P. & KEYS, H. E. (1956). Blood levels of 17-hydroxycorticosteroids in hyperthermic dogs. *Proc. Soc. exp. Biol. Med.* **93**, 280–284.

BASS, D. E. & HENSCHEL, A. (1956). Responses of body fluid compartments to heat and cold. *Physiol. Rev.* **36**, 128–144.

BEISEL, W. R., GOLDMAN, R. F. & JOY, R. T. J. (1968). Metabolic balance studies during induced hyperthermia in man. *J. appl. Physiol.* **24**, 1–10.

BLIGH, J. (1966). The thermosensitivity of the hypothalamus and thermoregulation in mammals. *Biol. Rev.* **41**, 317–367.

BROWN, H., ENGLERT, E., WALLACH, S. & SIMONS, E. L. (1957). Metabolism of free and conjugated 17-hydroxycorticosteroids in normal subjects. *J. clin. Endocr. Metab.* **17**, 1191–1201.

BUSH, I. E. (1952). Methods of paper chromatography of steroids applicable to the study of steroids in mammalian blood and tissue. *Biochem. J.* **50**, 370–378.

BUSH, I. E. (1962). Chemical and biological factors in the activity of adrenocortical steroids. *Pharmac. Rev.* **14**, 317–445.

CHOWERS, I., HAMMEL, H. T., EISENMAN, J., ABRAMS, R. M. & MCCANN, S. M. (1966). Comparison of effect of environmental and pre-optic heating and pyrogen on plasma cortisol. *Am. J. Physiol.* **210**, 606–610.

COLLINS, K. J., FEW, J. D., FORWARD, T. J. & GIEC, L. A. (1968). The plasma glucocorticoid response to environmental heat stress. *J. Physiol.* **194**, 33–34*P*.

COLLINS, K. J. & WEINER, J. S. (1968). Endocrinological aspects of exposure to high environmental temperatures. *Physiol. Rev.* **48**, 785–839.

FALBRIARD, A., MULLER, A. F., NEHER, R. & MACH, R. S. (1955). Etude des variations de l'aldosteronure et de deperditions renale et extrarenales de sel et d'eau. *Schweiz. med. Wschr.* **85**, 1218–1220.

FEW, J. D. (1961). A method for the analysis of urinary 17-hydroxycorticosteroids. *J. Endocr.* **22**, 31–46.

FEW, J. D. (1968). A simple method for the separate estimation of 11-deoxy and 11-oxygenated 17-hydroxycorticosteroids in human urine. *J. Endocr.* **41**, 213–222.

FEW, J. D. & COLLINS, K. J. (1969). Diurnal variation in the steroid 11-oxygenation index. *J. Endocr.* (In the Press.)

FEW, J. D. & FORWARD, T. J. (1968). The quantitative thin layer chromatography of corticosteroids. *J. Chromat.* **36**, 63–73.

FINDLAY, J. D. (1957). The respiratory activity of calves subjected to thermal stress. *J. Physiol.* **136**, 300–309.

FLOOD, C., LAYNE, D. S., RAMCHARAN, S., ROSSIPAL, E., TAIT, J. F. & TAIT, S. A. S. (1961). An investigation of the urinary metabolites and secretion rates of aldosterone and cortisol in man and a description of methods for their measurement. *Acta endocr., Copenh.* **36**, 237–264.

FOX, R. H. (1967). Further development of the thermoregulatory function test. In *Comparative Methodology for Heat Tolerance Testing*, ed. HENSCHEL, A., pp. 267–328. U.S. Public Health Service Rpt. TR-44,

HALE, H. B., SAYERS, G., SYDNOR, K. L., SWEAT, M. L. & VAN FOSSAN, D. D. (1957). Blood adrenocorticotrophic hormone and plasma corticoids in men exposed to adverse environmental conditions. *J. clin. Invest.* **36**, 1642–1646.

HELLMANN, K., COLLINS, K. J., GRAY, C. H., JONES, R. M., LUNNON, J. B. & WEINER, J. S. (1956). The excretion of urinary adrenocortical steroids during heat stress. *J. Endocr.* **14**, 209–216.

ICHIKAWA, Y. (1966). Metabolism of cortisol-4-C^{14} in patients with infectious and collagen disease. *Metabolism* **15**, 613–625.

INGRAM, D. L., MCLEAN, J. A. & WHITTOW, G. C. (1961). Increase of evaporative heat loss of water from the skin of the ox in response to local heating of the hypothalamus. *Nature, Lond.* **191**, 81–82.

JENKINS, J. S. & SAMPSON, P. A. (1967). Conversion of cortisone to cortisol and prednisone to prednisolone. *Br. med. J.* **2**, 205–207.

MACFARLANE, W. V. (1963). Endocrine functions in hot environments. In *Environmental Physiology and Psychology in Arid Conditions, Reviews of Research*, vol. 22, pp. 153–222. Paris: UNESCO.

OKITA, G. T., KABARA, J. J., RICHARDSON, F. & LE ROY, G. V. (1957). Assaying Compounds containing H^3 and C^{14}. *Nucleonics* **15**, 111–114.

PROVINS, K. A., HELLON, R. F., BELL, C. R. & HIORNS, R. W. (1962). Tolerance to heat of subjects engaged in sedentary work. *Ergonomics* **5**, 93–97.

RICHARDS, J. B. & EGDAHL, R. H. (1956). Effect of acute hyperthermia on adrenal 17-hydroxycorticosteroid secretion in dogs. *Am. J. Physiol.* **186**, 435–439.

ROBINSON, K. W. & MACFARLANE, W. V. (1958). Urinary excretion of adrenal steroids during exercise in hot atmospheres. *J. appl. Physiol.* **12**, 13–16.

STREETEN, D. H. P., CONN, J. W., LOUIS, L. W., FAJANS, S. S., SELTZER, H. S., JOHNSON, R. D., GITTLER, R. D. & DUBE, A. H. (1960). Secondary aldosteronism: metabolic and adrenocortical responses of normal men to high environmental temperatures. *Metabolism* **9**, 1071–1092.

THORN, G. W., JENKINS, D. & LAIDLAW, J. C. (1953). The adrenal response to stress in man *Recent Prog. Horm. Res.* **8**, 171–215.